

and diafiltered vs. 3.5 vol 0.1 M Na<sub>2</sub>CO<sub>3</sub>, pH 10.2, then vs. 3.5 vol 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.2. The filter-sterilized enzyme was stable at 4° for at least one month.

## 5 Table 4.

### Summary of Purification and PEGylation of Recombinant Pig-Baboon Chimeric (PBC) Uricase

A. Purification Fraction	Total protein	Total uricase activity	Specific activity	Recovery of activity
	mg	μmol/min	μmol/min/mg	%
Crude extract	1565	1010	0.6	100
Q-Sepharose	355	1051	3.0	104
Sephacryl S-200	215	1170	5.5	116
<b>B. PEGylation</b>				
S-200 uricase	100	546	5.5	100
PEG-uricase	97	336	3.5	62

10

Figure 1 shows a SDS-mercaptoethanol PAGE (12% gel) analysis of fractions obtained during the purification and PEGylation of recombinant pig-baboon chimera (PBC) uricase. Lanes: 1= MW markers; 2= SDS extract of uninduced pET3d-PBC cDNA-transformed cells (*E. coli* BL21(DE3)pLysS); 3= SDS extract of IPTG-induced pET-PBC cDNA-transformed cells; 4= Crude extract (see Table 5); 5= concentrated Q-sepharose uricase pool; 6= concentrated Sephacryl S-200 uricase pool; 7= PEGylated Sephacryl S-200 recombinant PBC uricase.

15

20

The results shown in Table 4 show that the purified PBC uricase could be modified with retention of about 60% of catalytic activity. In this PEGylation reaction all of the uricase subunits were modified (Figure 1, lane 7). In studies not shown, the PEGylated enzyme had similar kinetic properties to unmodified PBC uricase ( $K_M$  10-20

$\mu\text{M}$ ). Importantly, the modified enzyme was much more soluble than the unmodified enzyme at physiologic pH ( $>5$  mg/ml in PBS vs.  $<1$  mg/ml). The PEGylated enzyme could also be lyophilized and then reconstituted in PBS, pH 7.2, with minimal loss of activity. In other experiments, we compared the activities of this preparation of PEG-PBC uricase with the *A. flavus* uricase clinical preparation. At pH 8.6 in borate buffer, the *A. flavus* enzyme had 10-14 fold higher  $V_{\text{max}}$  and a 2 fold higher  $K_{\text{M}}$ . However, in PBS, pH 7.2, the PEG-PBC and unmodified fungal enzymes differed in uricase activity by  $<2$  fold.

#### 10 EXAMPLE 4

##### Circulating life in mice of unmodified and PEGylated PBC uricase.

Figure 2 shows the circulating life of native and PEGylated PBC uricase. Groups of mice (3 per time point) were injected IP with 1 unit of native (circles) or PEG-modified (squares) recombinant PBC uricase (preparation described in Example 3). At the indicated times, blood was obtained from sets of three mice for measuring serum uricase activity. The PEGylated uricase (described in Example 3) had a circulating half-life of about 48 hours, vs.  $<2$  hours for the unmodified enzyme (Fig 2).

20

#### EXAMPLE 5

##### Efficacy of PEGylated uricase of invention.

Figure 3 shows the relationship of serum uricase activity to the serum and urine concentrations of uric acid. In this experiment, a homozygous uricase-deficient knockout mouse (Wu et al. 1994) received two injections, at 0 and 72 hours, of 0.4 IU of recombinant PBC uricase that had been PEGylated. The uricase deficient knock-out mouse was used in this experiment because, unlike normal mice that have uricase, these knock-out mice, like humans, have high levels of uric acid in their blood and body

30

fluids and excrete high levels of uric acid in their urine. These high levels of uric acid cause serious injury to the kidneys of these mice, which is often fatal (Wu et al. 1994).

The experiment shown in Figure 3 demonstrates that intraperitoneal injections of a PEGylated preparation of recombinant PBC uricase resulted in an increase in serum uricase activity, which was accompanied by marked decline in the serum and urinary concentrations of uric acid in a uricase-deficient mouse.

#### EXAMPLE 6

##### 10 Nonimmunogenicity of construct-carrier complex

PEGylated recombinant PBC uricase was injected repeatedly into homozygous uricase-deficient mice without inducing accelerated clearance, consistent with absence of significant immunogenicity. This was confirmed by ELISA. Figure 4 shows maintenance of circulating levels of uricase activity (measured in serum) after repeated injection. PEGylated PBC uricase was administered by intraperitoneal injection at 6-10 day intervals. Serum uricase activity was determined 24 hours post injection.

#### EXAMPLE 7

20

##### Covalent linkage to mutationally introduced lysine

PEGylation of purified recombinant PBC uricase should result in attachment of PEG to the novel lysine (residue 291). In this experiment a preparation of PBC uricase could be modified by PEGylation. It can be determined by means known in the art whether the peptide containing the novel lysine (residue 291) has been modified by PEGylation.

##### 30 REFERENCES